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Letter to the Editor

Additional complexity in p73: induction by mitogens in lymphoid cells and identification of two new splicing variants ε and ζ

Dear Editor,

p53 is a sequence specific transcription factor which transactivates several genes important in the apoptotic pathway, such as p21, mdm2, gadd45, bax and caspases.[1,2] Lack of apoptosis, with inappropriate cell proliferation in cancer is correlated with a high frequency of p53 mutations, in some tumours reaching 50%. Perhaps surprisingly, in view of the importance of p53 in regulating cell death and its strong phylogenetic conservation, only recently have homologous genes been identified.

These (p63 and p73) show up to 63% aminoacid identity with p53 in the DNA binding, oligomerization and transcription activation domains, suggesting a similar mechanism of action to p53.[3–7] p63 is expressed as six different forms.[4] These use one of the two alternative transcription initiation sites, each transcript then being expressed as one of three alternatively spliced variants (α, β, γ). Since the transcripts using the downstream ATG lack the first three exons coding for the transactivation domain, they act as natural dominant negative mutants of full length p63 and of p53.[4]

The p73 gene comprises 14 exons and we have shown previously that in addition to the full length α form and the alternatively spliced transcript lacking exon 13 (β) other splice variants, lacking exon 11 (γ) and exons 11, 12 and 13 (δ) are also produced.[8] Stimulation of the T lymphoblastoid cell line, Jurkat, and human peripheral blood lymphocytes (PBL) with phytoemagglutinin (PHA) causes a 3 and 2.6-fold increase respectively in p73 expression by Northern blotting after 24 h (panel A). This is associated with induction of 34.7% and 21.2% of apoptotic cells respectively. No p53 was detected in Jurkat cells after PHA treatment (not shown).

In order to discriminate the differential induction of the four p73 isoforms we performed an RT–PCR using isoform-specific primers on RNA extracted from cells treated under the same conditions. Panel B shows upregulation of α, β, γ and δ in PHA-treated PBL and Jurkat cells. Densitometric comparison of these with the housekeeping gene GAPDH showed that the increase in expression of each isoform were comparable (not shown).

In addition, a new isoform was amplified from normal PBL. Cloning and sequencing of this p73ε identified it as a splicing variant lacking exons 11 and 13 (panel C). To confirm the existence of p73ε, we screened a panel of normal and tumour cell lines. As also shown in panel B, p73ε was also present in the human hepatoma line HepG2, and a sixth isoform ζ was identified in the MCF7 human breast cancer cell line and in a human skin biopsy. p73 ζ is a further splice variant which lacks exons 11 and 12, and results in the loss of 96 aminoacids, the sequence continuing with the C-terminus of the α form (panel C). In p73ε, loss of exon 11 deletes 50 aminoacids with a frame shift to the reading frame of the γ isoform; splicing of exon 13 deletes an additional 31 aminoacids and reverts the reading frame to the α variant.

The p73 isoforms are shown schematically in panel C.

It is tempting to speculate that these six p73 isoforms interact with each other to regulate p73 function. Indeed, p73 α, β, γ and δ show dramatic differences in their ability to homo/heterodimerise and transactivate p21, associated with differences in colony formation after overexpression in SAOS2 cells.[8] The data suggest that the six splice variants of p73, together with those of p63, may also interact with each other and with p53 in a complex network that regulates cell growth, differentiation and death.

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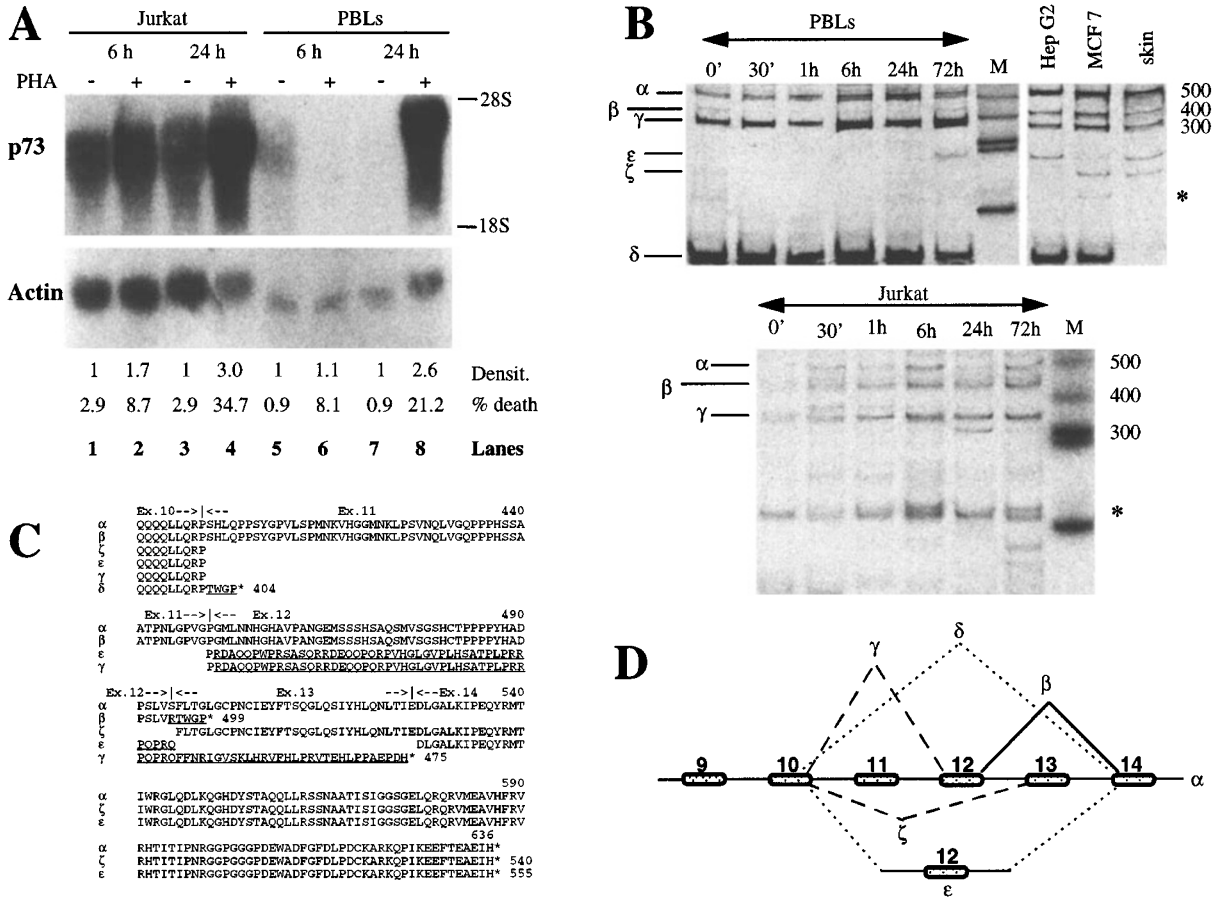

Figure 1 Modulation of p73 and its six splicing variants in human lymphoid cells. (**A**) Northern blot analysis of p73 in Jurkat and PBL cells. Total RNA (20 μg) from untreated or PHA-treated [PHA-M (DIFCO) 1% v/v] cells were electrophoresed and hybridized with a specific random priming ^{32}P probe (s.a.: 1×10^9 c.p.m. μg^{-1}). Densitometry (arbitrary units) was normalized for actin expression. The corresponding induction of PCD (% of apoptotic events evaluated by propidium idodide staining visualized by FACS-Calibur, Becton DIckenson) is shown below each lane. (**B**) Expression of p73 splicing variants by RT–PCR in lymphoid (Jurkat and PBLs) and non lymphoid cells (human skin biopsy, HepG2 and MCF7). RT–PCR was performed as described.[8] Two new isoforms (ε, ζ) were detected in addition to the four previously identified.[8] *Denotes new, yet uncharacterized, isoform detected only in transformed cells (MCF7 and Jurkat). (**C**) Amino acid alignment of the C-terminal region of the different p73 splicing variants. The corresponding exons are indicated. Underlined sequences represent a different reading frame within the splicing variants. (**D**) Schematic representation of the four p73 splicing variants α, β, γ, δ previously described[8] and of the two new isoforms p73 ε, ζ described in the present report.